altered, the biological activity is substantially retained. Further, the invention also includes mutants of organisms hosting all or part of a δ-endotoxin encoding a gene of the invention. Such mutants can be made by techniques well known to persons skilled in the art. For example, UV irradiation can be used to prepare mutants of host organisms. Likewise, such mutants may include asporogenous host cells which also can be prepared by procedures well known in the art.

5.0 EXAMPLES

The following examples are included to demonstrate preferred embodiments of the invention. It should be appreciated by those of skill in the art that the techniques disclosed in the examples which follow represent techniques discovered by the inventor to function well in the practice of the invention, and thus can be considered to constitute preferred modes for its practice. However, those of skill in the art should, in light of the present disclosure, appreciate that many changes can be made in the specific embodiments which are disclosed and still obtain a like or similar result without departing from the spirit and scope of the invention.

5.1 EXAMPLE 1 -- PREPARATION OF TEMPLATES FOR RANDOM MUTAGENESIS

Structural maps for the *cry1C* plasmids pEG315 and pEG916 are shown in FIG. 2. The *cry1C* gene contained on these plasmids was isolated from the *B. thuringiensis* strain EG6346 subsp. *aizawai*, first described by Chambers *et al.* (1991). An ~4 kb *Sal*I-*Bam*HI fragment containing the intact *cry1C* gene from EG6346 was cloned into the unique *Xho*I and *Bam*HI sites of the shuttle vector pEG854, described by Baum *et al.* (1990) to yield pEG315. pEG916 is a pEG853 derivative (also described by Baum *et al.*, 1990) containing the same *cry1C* gene fragment and a 3′ transcription terminator region derived from the *cry1F* gene described by Chambers *et al.* (1991).

pEG345 (FIG. 3) is a pEG597 derivative (also described by Baum *et al.*, 1990) that contains the *cry1C* gene from *B. thuringiensis* subsp. *aizawai* strain 7.29, described by Sanchis *et al.* (1989) and disclosed in the European Pat. Appl. No. EP 295156A1 and

Intl. Pat. Appl. Publ. No. WO 88/09812. Both genes are nearly identical to the holotype *cryIC* gene described by Honee *et al.* (1988).

The recombinant DNA techniques employed are familiar to those skilled in the art of manipulating and cloning DNA fragments and employed pursuant to the teachings of Maniatis *et al.* (1982) and Sambrook *et al.* (1989).

A frame-shift mutation was introduced into the *cryIC* gene of pEG916 at codon 118. By analogy to the published crystal structures for Cry1Aa and Cry3A, the glutamic acid residue (E) at this position is predicted to lie within or immediately adjacent to the loop region between α helices 3 and 4 of Cry1C domain 1, the target site for random mutagenesis. This mutated gene can be used as a template for oligonucleotide-directed mutagenesis using a mutagenic primer that corrects the frame-shift mutation, thus ensuring that the majority of clones recovered encoding full-length protoxin molecules will have incorporated the mutagenic oligonucleotide.

The frame-shift mutation was introduced by a PCR™-mediated mutagenesis protocol using the oligonucleotide primers A, B, and C and pEG916 (FIG. 2) as the DNA template. The mutagenesis protocol, described by (Michael, 1994) relies on the use of a thermostable ligase to incorporate a phosphorylated mutagenic oligonucleotide into an amplified DNA fragment. The DNA sequence of these primers is shown below:

Primer A: (SEQ ID NO:15)

5'-CCCGATCGGCCGCATGC-3'

Primer B: (SEQ ID NO:16)

5'-GCATTTAAAGAATGGGAAGGGATCCTAGGAATCCAGCAACCAGGACCAGAG-3'

Primer C: (SEQ ID NO:17)

5'-GAGCTCTTGTTAAAAAAGGTGTTCCAGATC-3'

The mutagenic oligonucleotide, primer B, was designed to incorporate a *Bam*HI and *Bln*I restriction site in addition to the frame-shift mutation at codon 118 (FIG. 4). The product obtained from the PCR™ was resolved by electrophoresis of an agarose-TAE gel and purified using the Geneclean II® Kit (Bio 101, Inc., La Jolla, CA) following the manufacturer's suggested protocol. The purified DNA fragment was digested with the restriction enzymes *Age*I and *Bbu*I. pEG916 was also digested with the restriction

enzymes *Age*I and *Bbu*I and the restricted DNA fragments resolved by agarose gel electrophoresis and the vector fragment purified as described above. The amplified DNA fragment and the pEG916 vector fragment were ligated together with T4 ligase, and the ligation reaction used to transform the acrystalliferous *B. thuringiensis* strain EG10368 (described in U. S. Patent 5,322,687) to Cml resistance, using the electroporation procedure described by Mettus and Macaluso (1990). Individual transformants were selected and many were determined to be acrystalliferous by phase-contrast microscopy of the sporulated cultures. Recombinant plasmids were isolated from *B. thuringiensis* transformants using the alkaline lysis procedure described by Maniatis *et al.* (1982). Incorporation of the frame-shift mutation into *cry1C* was also indicated by the presence of the *Bam*HI and *Bln*I sites, determined by restriction enzyme analysis of the recombinant plasmids isolated from the EG10368 transformants. The recombinant plasmid incorporating the frame-shift mutation and the *Bam*HI and *Bln*I sites was designated pEG359 (FIG. 2 and FIG. 4).

pEG359 was introduced into the *E. coli* host strain DH5α by transformation using frozen competent cells and procedures obtained from GIBCO BRL (Gaithersburg, MD). pEG359, purified from *E. coli* using the alkaline lysis procedure (Maniatis *et al.*, 1982), was further modified by digestion with the restriction enzyme *Bgl*II and religation of the vector fragment with T4 ligase. The ligation reaction was used to transform the *E. coli* host strain DH5α as before. The resulting plasmid, designated p154 (FIG. 2), contains a deletion of the *cry1C* gene sequences downstream of the unique *Bgl*II site in *cry1C*.

5.2 EXAMPLE 2 -- RANDOM MUTAGENESIS OF NUCLEOTIDES 352-372 IN *CRY1C*

Mutagenesis of nucleotides 352-372, encoding the putative loop region between α helices 3 and 4 of Cry1C domain 1, was performed according to the PCR™-mediated "Megaprimer" method as described (Upender *et al.*, 1995), using the oligonucleotide primers A (SEQ ID NO:15), C (SEQ ID NO:17), and D (SEQ ID NO:18).

Primer D: (SEQ ID NO:18)

5'-GCATTTAAAGAATGGGAANNNNNNNNNNNNNNNNNNNNNACCAGGACCAGAGTAATTGATCGC-3'

N (20, 21, 23, 28, 29, 31, 32, and 39) = 82% A; 6% G, C, T,